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(54) Title: CYCLIZED FLUORESCENT DNA-INTERCALATING CYANINE DYES

(57) Abstract

New intercalating asymmetric cyanine dyes are provided in which the benzothiazole portion of the cyanine dye has been modified to produce dyes with improved properties for labelling nucleic acids, such as longer wavelengths and improved fluorescence enhancement when bound to DNA or RNA. More specifically, the dyes are cyclized fluorescent cyanine dyes for non-covalently labelling nucleic acids. Methods are described for detecting nucleic acids in a sample by contacting the nucleic acids with a fluorescent cyanine dye and monitoring the change in fluorescence emission of the dye.

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Title: CYCLIZED FLUORESCENT DNA-INTERCALATING CYANINE DYES

FIELD OF THE INVENTION

The present invention relates generally to dyes for labelling nucleic acids. More specifically, the present invention relates to intercalating cyanine dyes for the detection and enumeration of nucleic acids.

BACKGROUND OF THE INVENTION

Intercalating dyes which exhibit enhanced fluorescence upon binding to DNA or RNA are a basic tool in molecular and cell biology. In general, intercalating dyes bind noncovalently to DNA through a combination of hydrophobic interactions with the DNA base-pairs and ionic binding to the negatively charged phosphate backbone. The fluorescence of the dye is ideally increased several-fold upon binding to DNA, thereby enabling the detection of small amounts of nucleic acids. Examples of fluorescent noncovalent DNA binding dyes include ethidium bromide which is commonly used to stain DNA in agarose gels after gel electrophoresis, and propidium iodide and Hoechst 33258 which are used in flow cytometry to determine the DNA ploidy of cells.

Fluorescent nucleic acid labelling dyes preferably absorb light between about 300 and 900 nm and preferably have a Stokes shift of at least about 10 nm. Dyes that absorb light in the 500 to 900 nm range are preferred because they are spectrally removed from other components that may be present in a biological sample and because they may be used with inexpensive light sources. Fluorescent dyes that have a high extinction coefficient, a high quantum yield, and significantly enhanced fluorescence when bound to a nucleic acid are also preferred.

Few new dye chromophores were described until the introduction of Thiazole Orange as a reticulocyte stain in 1986. Lee, et al., "Thiazole Orange: A New Dye for Reticulocyte Analysis", Cytometry **1986** 7, 508-517. Thiazole

Orange is an asymmetric cyanine dye. Although many asymmetric cyanine dyes have been described in the art (e.g., Lincoln, et al., U.S. Patent No. 3,282,932), Thiazole Orange's fluorescence properties when bound to DNA and RNA and its utility for labelling nucleic acids had not been previously identified. Lee, et al., U.S. Patent No. 4,957,870. For example, unlike most asymmetric cyanine dyes, Thiazole Orange exhibits a several thousand-fold enhancement in fluorescence upon binding to DNA.

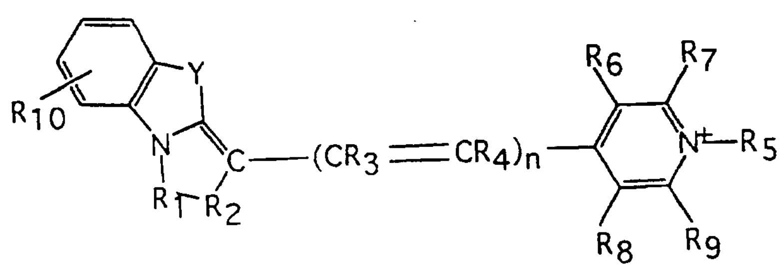
Since the discovery of Thiazole Orange as a nucleic acid dye, several improvements to Thiazole Orange and its trimethine homologs have been developed to provide dyes with tighter binding to DNA and greater water solubility. Xue, et al. U.S. Patent No. 5,321,130 and Glazer, et al. U.S. Patent No. 5,312,921. These dyes generally involve a modification to the quinolinium portion of the dye.

A continuing need exists for new and improved dyes for labelling nucleic acids. In particular, a need exists for dyes which exhibit longer wavelengths and significantly enhanced fluorescence when bound to DNA or RNA.

SUMMARY OF THE INVENTION

The present invention relates asymmetric cyanine dyes for non-covalently labelling nucleic acids in which the benzothiazole portion of the dye has been modified to provide improved physical properties to the dye, such as longer wavelengths and improved fluorescence enhancement when bound to DNA or RNA.

More specifically, the invention relates to cyclized fluorescent cyanine dyes for non-covalently labelling nucleic acids. The cyclized fluorescent cyanine dyes according to the present invention are represented by General Formula I



I

where:

n is 0, 1 or 2;

Y may be either S or O;

R_1 and R_2 are taken together to form a 5, 6, 7 or 8 membered ring;

R_3 and R_4 may each independently be either hydrogen, $C_1 - C_{10}$ alkyl, $C_1 - C_{10}$ alkoxy, or $C_1 - C_{10}$ alkylthio;

R_5 may be a $C_1 - C_{50}$ alkyl, preferably substituted with one or more polar substituents which preferably includes one or more positively charged atoms, or a cyclized fluorescent cyanine dye of the

present invention, i.e., where R_5 is a linker between two cyclized fluorescent cyanine dyes;

R_6 and R_7 may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most preferably a 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups;

R_8 and R_9 may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most preferably a 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups; and

R_{10} may be either H, C_{1-6} alkyl, C_1 - C_{10} alkoxy or a fused benzene.

As used above, alkyl and alkoxy refer to any substituent having a carbon backbone having the specified range of carbon atoms. The carbon backbone may form a straight chain, may be branched or may be cyclic. The alkyl and alkoxy groups may be optionally substituted by a wide variety of substituents including, for example, alcohols, amines, thiols, phosphates, halides, ethers, esters, ketones, aldehydes, carboxylic acids, amides, cycloalkyls, and aromatic rings.

The invention also relates to the composition of a cyanine dye according to the present invention non-covalently bound to a nucleic acid sequence, i.e., RNA or DNA, which enables the nucleic acid sequence to be analytically detected.

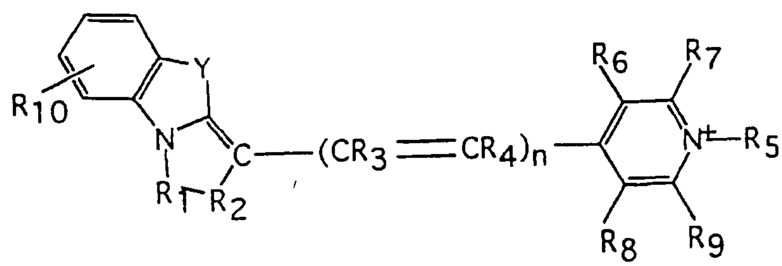
The invention also relates to a method for detecting nucleic acids in a sample by contacting the nucleic acids with a fluorescent cyanine dye according to the present invention and monitoring the change in fluorescence emission of the dye.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates asymmetric cyanine dyes for non-covalently labelling nucleic acids in which the benzothiazole portion of the dye

has been modified to provide improved physical properties to the dye, such as longer wavelengths and improved fluorescence enhancement when bound to DNA or RNA.

In one embodiment, the present invention relates to cyclized fluorescent cyanine dyes generally represented by General Formula I



I

where:

n is 0, 1 or 2;

Y may be either S or O;

R_1 and R_2 are taken together to form a 5, 6, 7 or 8 membered ring;

R_3 and R_4 may each independently be either hydrogen, C_1 - C_{10} alkyl, C_1 - C_{10} alkoxy, or C_1 - C_{10} alkylthio;

R_5 may be a C_1 - C_{50} alkyl, preferably substituted with one or more polar substituents which preferably includes one or more positively charged atoms, or a cyclized fluorescent cyanine dye of the present invention, i.e., where R_5 is a linker between two cyclized fluorescent cyanine dyes;

R_6 and R_7 may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most preferably a 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups;

R_8 and R_9 may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most preferably a 6

membered aromatic ring, optionally substituted with C_{1-8} alkyl or C_1 - C_{10} alkoxy groups; and

R_{10} may be either H, C_{1-8} alkyl, C_1 - C_{10} alkoxy or a fused benzene.

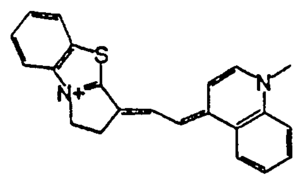
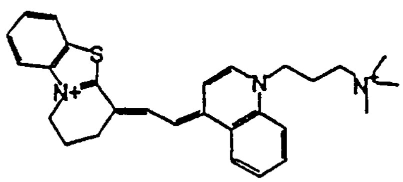
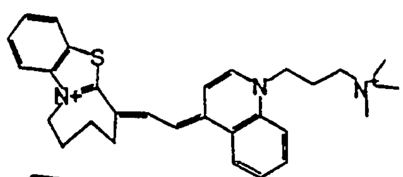
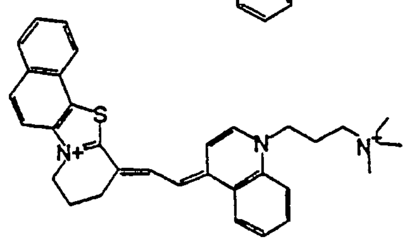
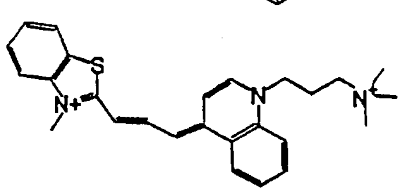
As used above, alkyl and alkoxy refer to any substituent having a carbon backbone having the specified range of carbon atoms. The carbon backbone may form a straight chain, may be branched or may be cyclic. The alkyl and alkoxy groups may be optionally substituted by a wide variety of substituents including, for example, alcohols, amines, thiols, phosphates, halides, ethers, esters, ketones, aldehydes, carboxylic acids, amides, cycloalkyls, and aromatic rings.

The cyclized cyanine dyes of the present invention provide the advantage over previous cyanine dyes of having higher absorbance and emission wavelengths. The cyclized cyanine dyes preferably absorb light at a wavelength of at least about 640 nm, more preferably at least about 649 nm and emit fluorescence at a wavelength of at least about 650 nm, more preferably at least about 663 nm. The cyclized cyanine dyes also preferably have a positive Stoke's shift ($\lambda_{Emission}-\lambda_{Abs.}$)of at least about 12 nm.

In particular, cyclized cyanine dyes having General Formula I where R_1 and R_2 are taken together to form a 5, 6, 7 or 8 membered ring have been found to absorb light and fluoresce when bound to a nucleic acid polymer at unexpectedly higher wavelengths than has been previously achieved by cyanine dyes where R_1 and R_2 do not form a ring structure.

Fluorescent cyanine dyes having the General Formula I where R_1 and R_2 are taken together to form a 7 membered ring have also been found to have the greatest Stoke's shift ($\lambda_{Emission}-\lambda_{Abs.}$)

TABLE 1: Absorbance and Emission Maxima of Intercalating Dyes in PBS with Excess DNA ([bp]/[dye] = 100)

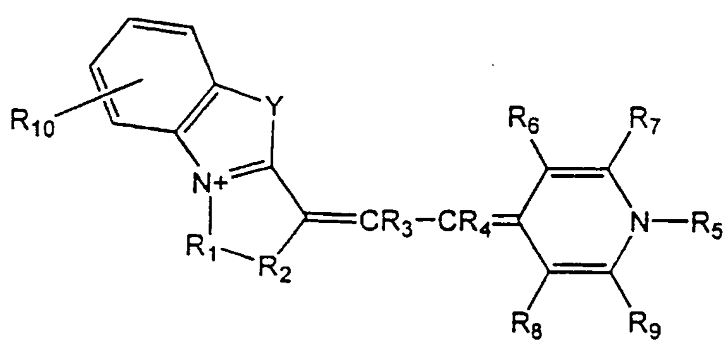
COMPOUND		Abs_{max}	Ems_{max}	F.E.
	1	649	663	100X
	2	654	667	100X
	3	654	672	30X
	4	675	690	200X
	5*	641	655	100X

Abs_{max}-Absorbance maximum (bounds to DNA)
Ems_{max}-Emission maximum (bound to DNA)
F.E.-fluorescence enhancement (bounds vs. not bound to DNA or RNA)
* Compound 5 is taught in U.S. Patent No. 5,321,130 to Yue, et al.

Table 1 summarizes the absorbance maximum and fluorescence emission maximum wavelengths (both when bound to DNA) of some exemplary cyclized cyanine dyes of the present invention.

As illustrated in Table 1, it was found that the addition of a cyclic aliphatic side chain to the basic cyanine dye structure, i.e., formation of a 5-8 membered ring by combining R_1 and R_2, was found to increase the absorbance and fluorescence emission wavelengths of the corresponding acyclic cyanine dye by about 12 nm. For example, as shown with regard to dyes **2** and **5**, dye 2 has an Abs_{max} at 654 nm as compared to 641 nm and an Ems_{max} at 667 nm as compared to 655 nm. In addition, dye **4** is the longest wavelength trimethine intercalating dye yet reported.

With regard to n, n may equal 1. Accordingly, the present invention includes cyclized cyanine dyes having the General Formula II (i.e. where n = 1)



II

where Y, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are as specified above.

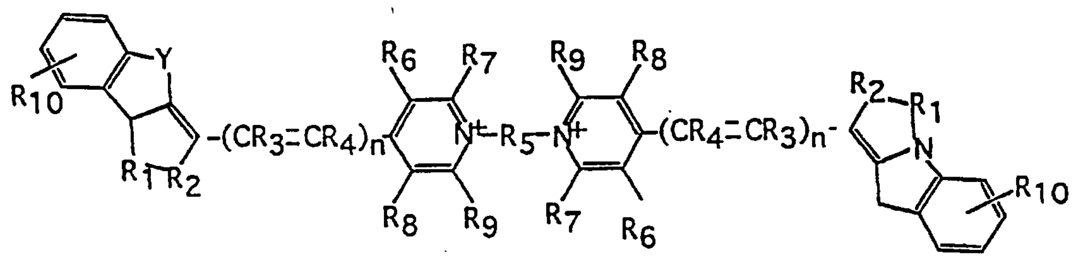
Y may be either S or O. and is most preferably S.

R_3 and R_4 may each independently be either hydrogen, C_1 - C_{10} alkyl, C_1 - C_{10} alkoxy, or C_1 - C_{10} alkylthio, and are preferably H.

R_5 may be a C_1 - C_{50} alkyl. Since DNA and RNA to which the cyclized cyanine dyes bind contain negatively charged phosphate backbones, it is preferred that R_5 be substituted with one or more polar substituents. It is most preferred that R_5 include one or more positively charged atoms in the polar

substituent. U.S. Patent No. 5,321,130 to Yue, et al. teaches unsymmetrical cyanine dyes having an aminoalkyl chain containing a backbone of 3-42 carbons and 1-5 positively charged nitrogen atoms. The cationic tail described in U.S. Patent No. 5,321,130 exemplifies one of the positively charged R_5 substituents that may be used in combination with the cyclic cyanine dyes of the present invention and is incorporated herein by reference. In addition to the positively charged R_5 substituents described in U.S. Patent No. 5,321,130, R_{12} is also intended to include aminoalkyl chains including a positively charged cyclic aminoalkyl group having 1-5 positively charged nitrogen atoms.

Alternatively, R_5 may form part of a linker between two cyclized fluorescent cyanine dyes as illustrated by General Formula IV



IV

According to this embodiment, Y, R_1, R_2, R_3, R_4, R_6, R_7, R_8, R_9 and R_{10} are as specified above. It should be noted that the two linked cyanine dyes may be the same or different cyanine dyes. In general, it is preferred that the linked cyanine dyes be the same since different dyes will have different spectral properties.

R_6 and R_7 may each independently be either H, C_{1-10} alkyl, or are taken together to form a 5 or 6 membered ring, most preferably a 5 or 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups.

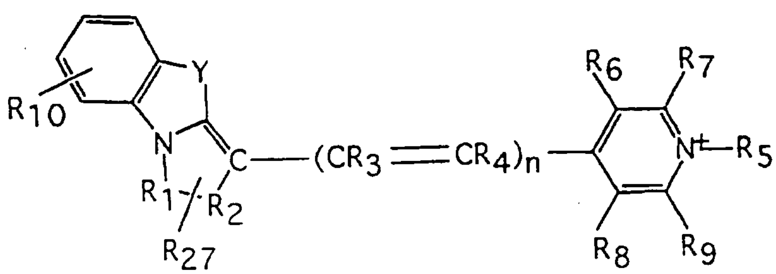
R_8 and R_9 may each independently be either H, C_{1-10} alkyl, or are taken together to form a 5 or 6 membered ring, most preferably a 5 or 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups.

In general, it is preferred either R_6 and R_7 or R_8 and R_9 are taken together to form a 5 or 6 membered aromatic ring, optionally substituted with

C_{1-6} alkyl or C_1 - C_{10} alkoxy groups. The R_6 and R_7 or R_8 and R_9 groups that do not form the aromatic ring are preferably H.

R_{10} may be either H, C_{1-6} alkyl, C_1 - C_{10} alkoxy or a fused benzene.

In a particularly preferred embodiment, the cyclized cyanine dye has the General Formula V where the ring formed by R_1 and R_2 includes a positively charged substituent R_{27}. As discussed herein, inclusion of a positively charged substituent, such as R_{27}, to a substituent on the positively charged nitrogen on the benzothiazole ring improves the net fluorescence enhancement of the dye with DNA.



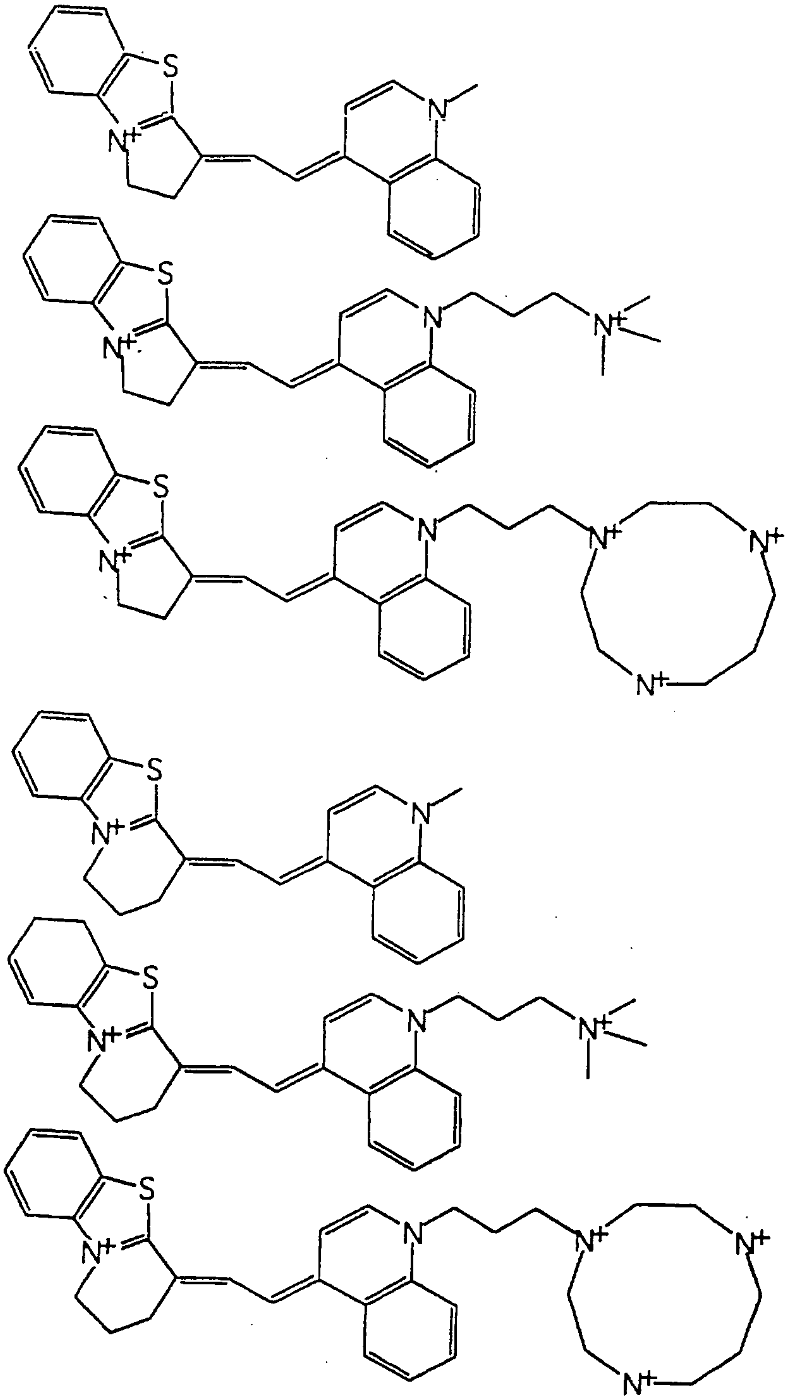
V

R_{27} is a positively charged alkyl substituent which may be attached to any atom used to form the 5, 6, 7 or 8 membered ring. R_{27} is more preferably a positively charged aminoalkyl substituent. For example, R_{12} can be an aminoalkyl chain containing a backbone of 3-42 carbons and 1-5 positively charged nitrogen atoms as described in U.S. Patent No. 5,321,130 to Yue, et al. which is incorporated herein by reference. In addition to the positively charged substituents described in U.S. Patent No. 5,321,130, R_{12} is also intended to include aminoalkyl chains including a positively charged cyclic aminoalkyl group having 1-5 positively charged nitrogen atoms.

In a preferred embodiment, R_{27} has the general formula $-R_{28}N(R_{29}R_{30}R_{31})$ where R_{28} is a C_{1-5} alkyl and R_{29}, R_{30}, and R_{31} are each independently a C_{1-10} alkyl.

Table 2 provides examples of some of the preferred cyclized cyanine dyes. It should be understood, however, that the dyes listed in Table 2 are intended only to exemplify the cyclized cyanine dyes of the present invention and are not intended to be limiting.

TABLE 2



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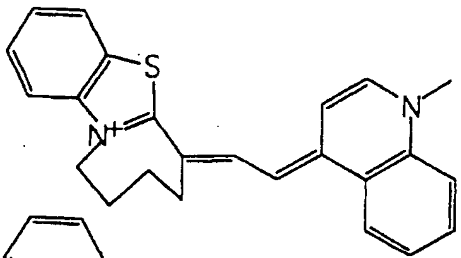

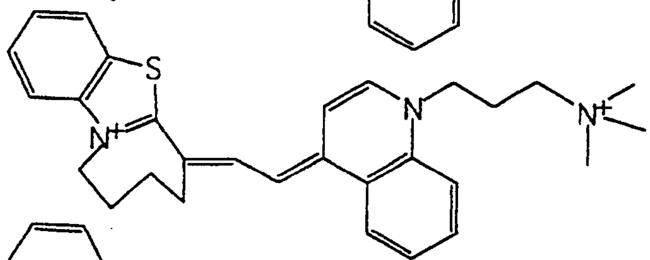

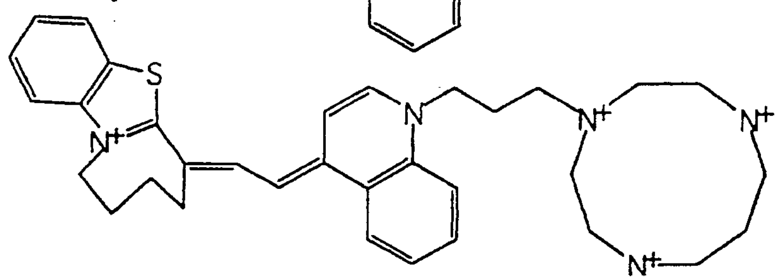

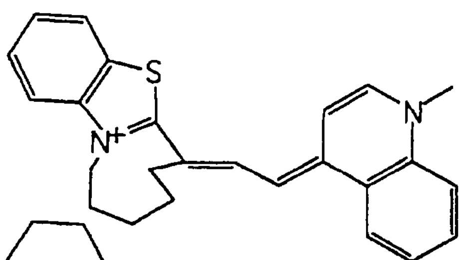

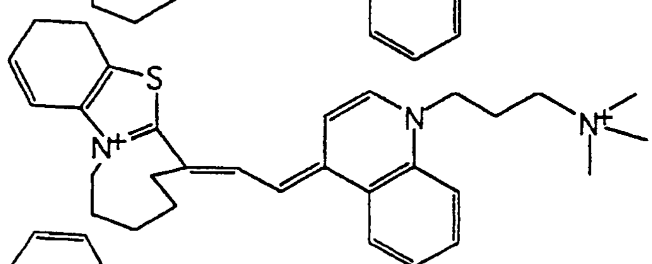

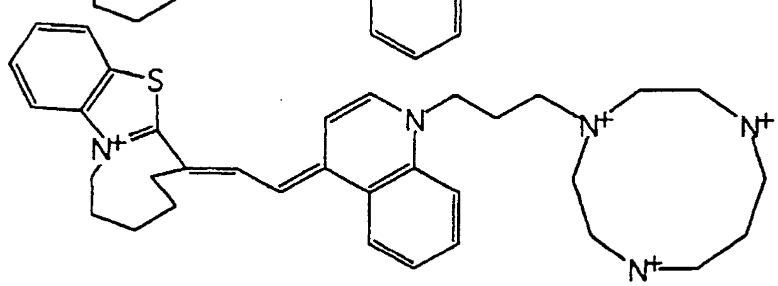

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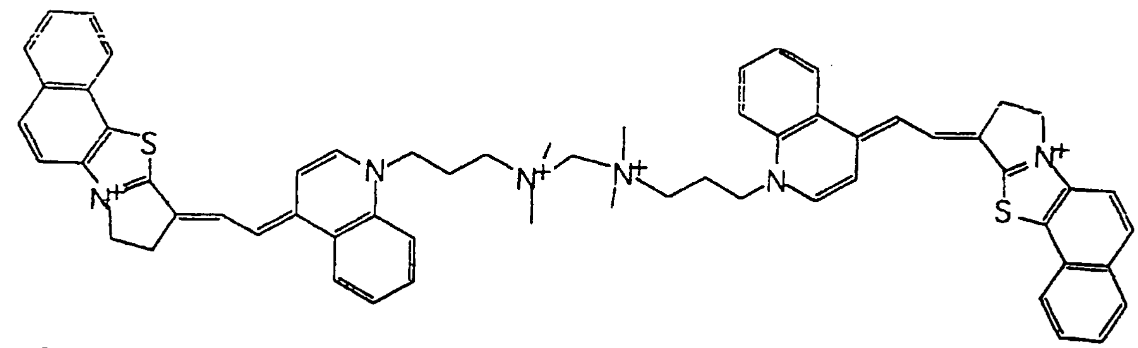

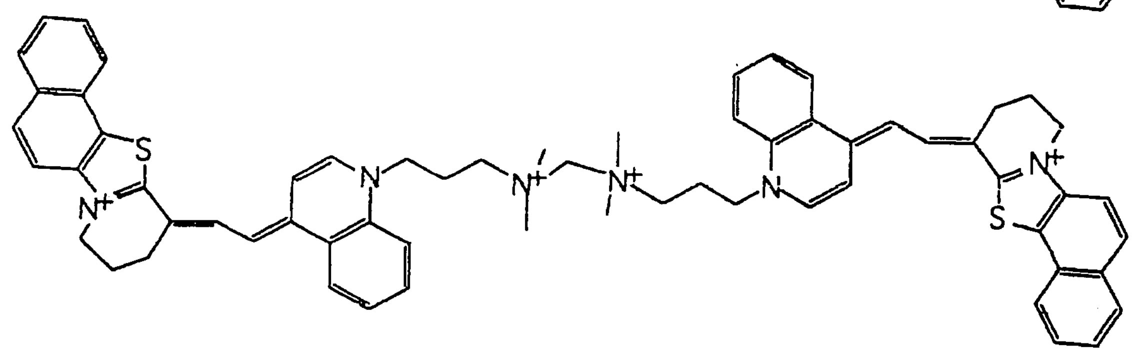

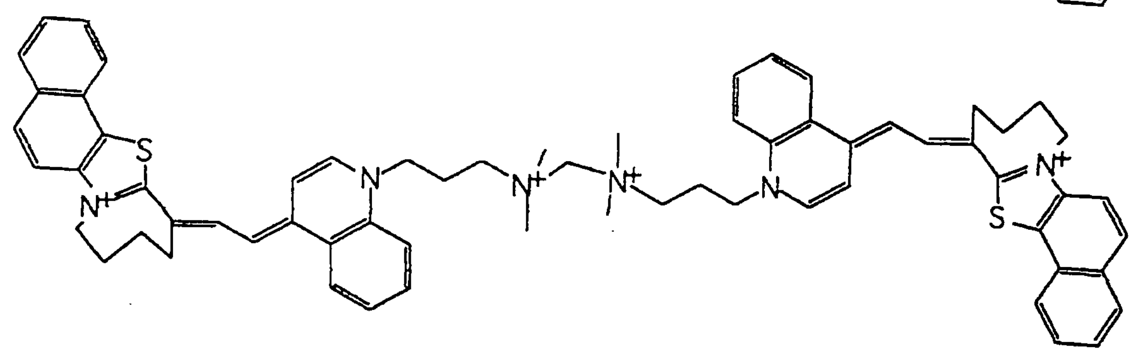

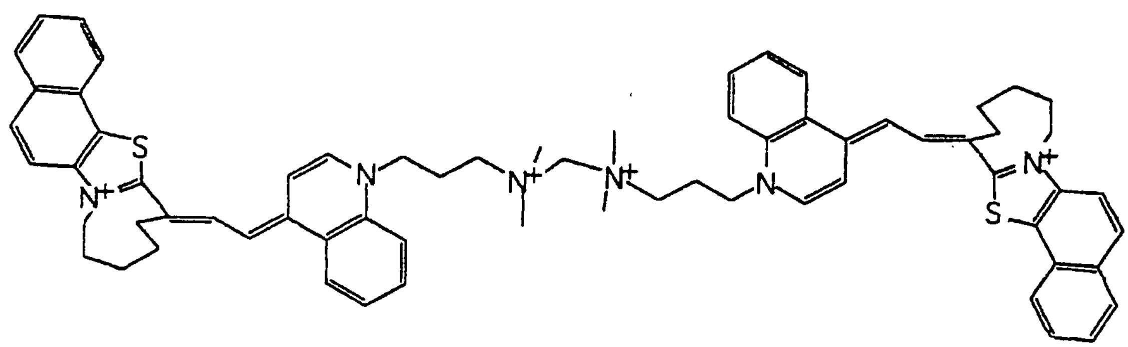

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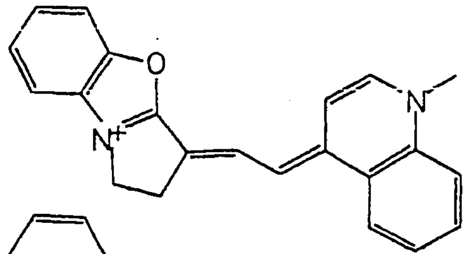

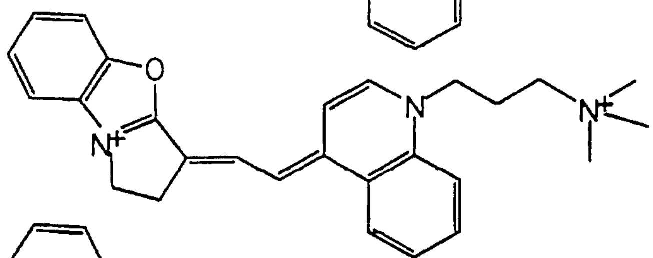

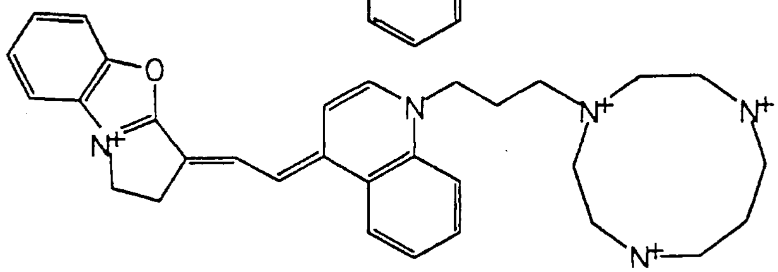

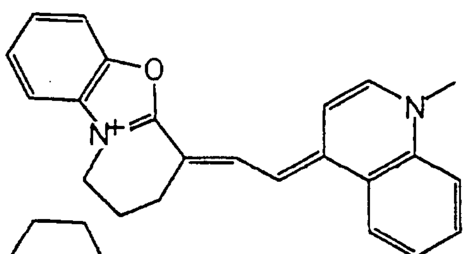

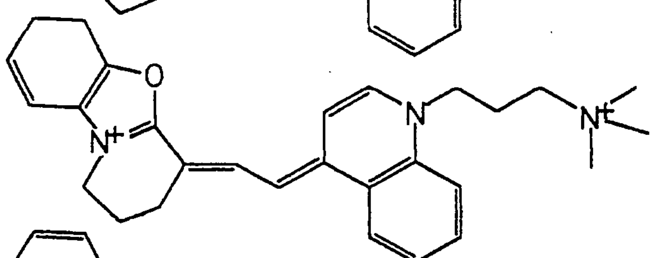

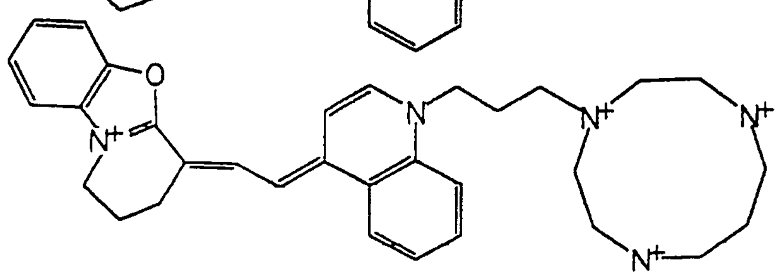

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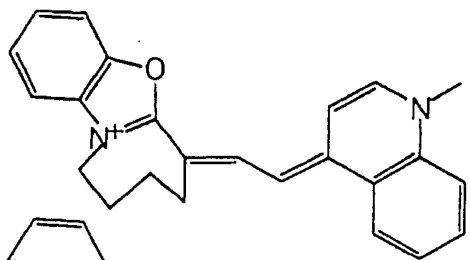
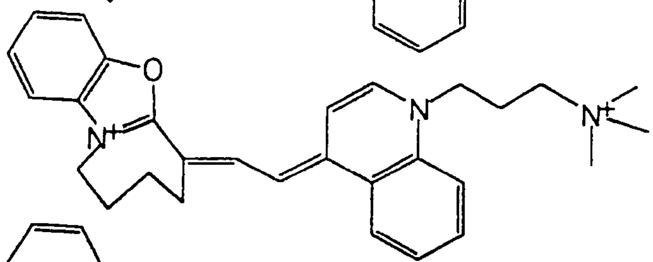
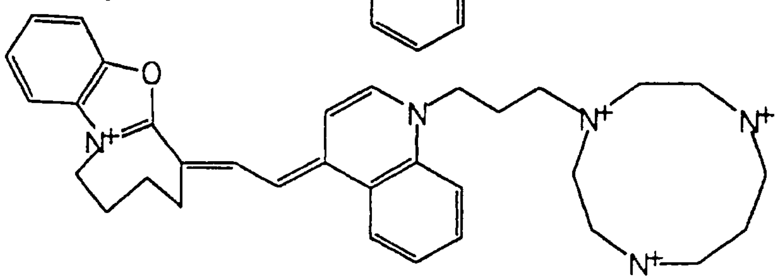
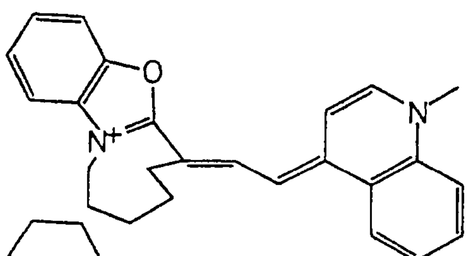
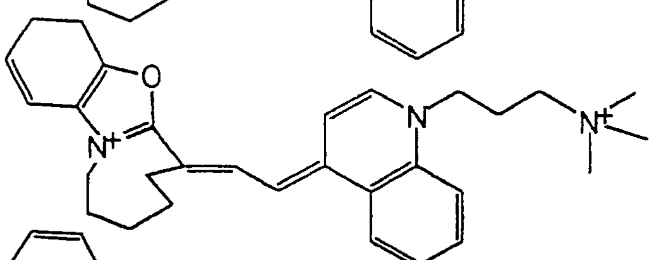










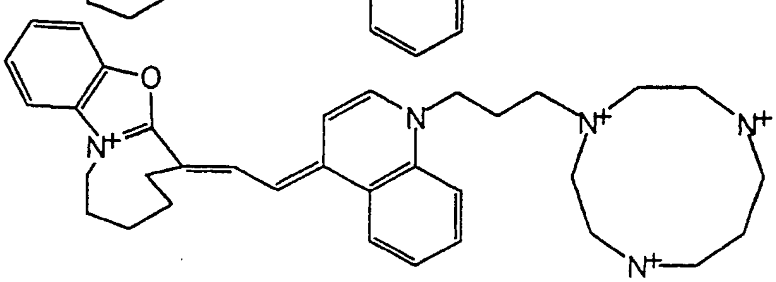
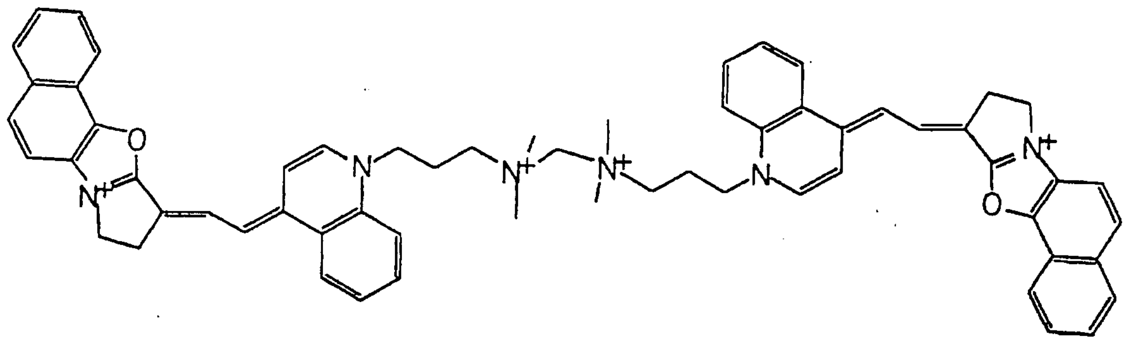
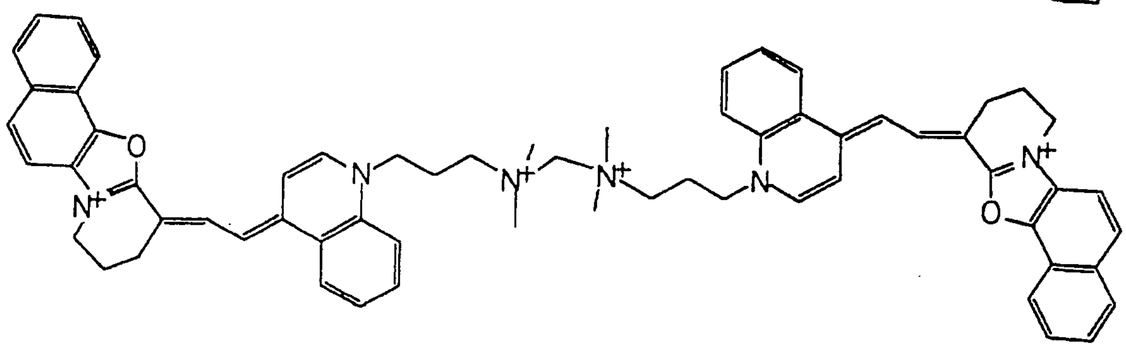
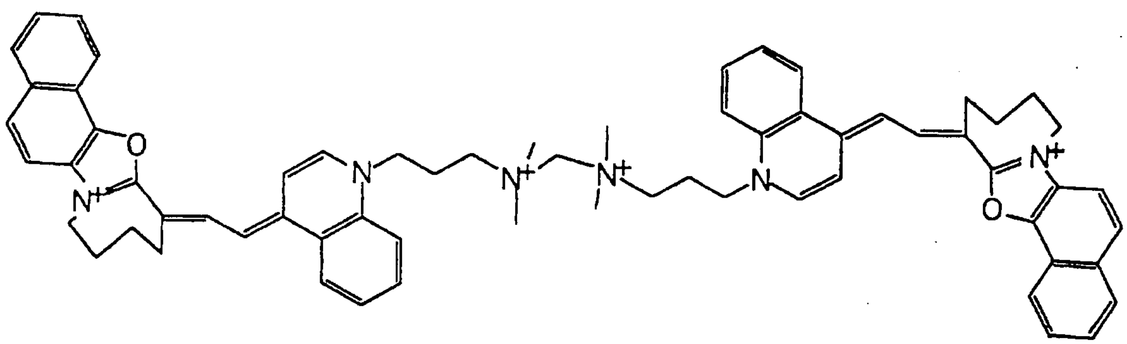
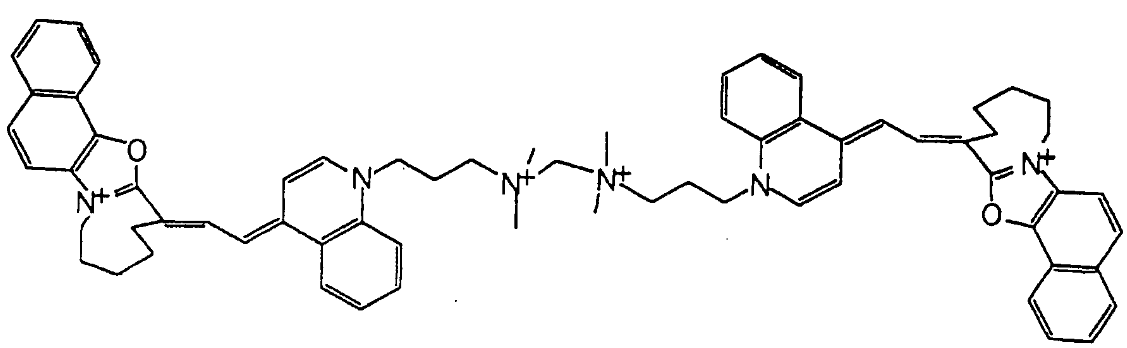


TABLE 2 (cont.)

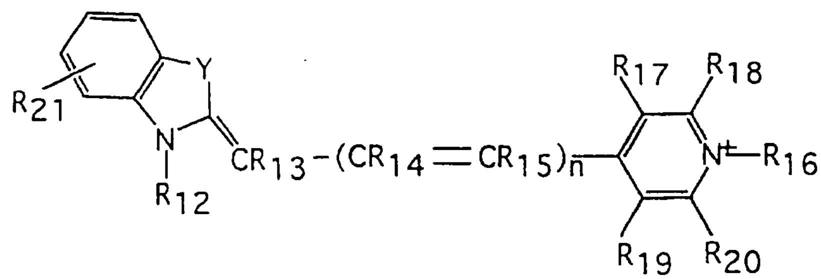








The present invention also relates to fluorescent cyanine dyes having a positively charged substituent attached to the positively charged nitrogen on the benzothiazole portion of the cyanine dye. These fluorescent cyanine dyes are represented by General Formula VI



VI

where

n is 0, 1 or 2;

Y may be either S or O;

R_{12} is a positively charged alkyl substituent, more preferably a positively charged aminoalkyl substituent;

R_{13}, R_{14} and R_{15} may each independently be either hydrogen, C_1 - C_{10} alkyl, C_1 - C_{10} alkoxy, or C_1 - C_{10} alkylthio;

R_{12} and R_{13} may optionally be taken together to form a 5, 6, 7 or 8 membered ring;

R_{16} may be a C_1 - C_{50} alkyl, preferably substituted with one or more polar substituents which preferably includes one or more positively charged atoms, or a cyclized fluorescent cyanine dye of the present invention, i.e., where R_{16} is a linker between two cyclized fluorescent cyanine dyes;

R_{17} and R_{18} may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most

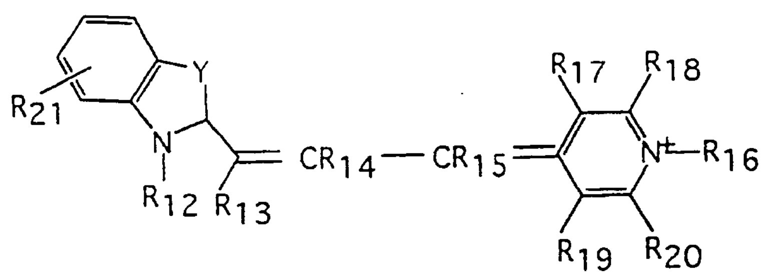
preferably a 5 or 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups;

R_{19} and R_{20} may each independently be either H or C_{1-10} alkyl, or may be taken together to form a 5 or 6 membered ring, most preferably a 5 or 6 membered aromatic ring, optionally substituted with C_{1-6} alkyl or C_1 - C_{10} alkoxy groups; and

R_{21} may be either H, C_{1-6} alkyl, C_1 - C_{10} alkoxy or a fused benzene.

As used above, alkyl and alkoxy refer to any substituent having a carbon backbone having the specified range of carbon atoms. The carbon backbone may form a straight chain, may be branched or may be cyclic. The alkyl and alkoxy groups may be optionally substituted by a wide variety of substituents including, for example, alcohols, amines, thiols, phosphates, halides, ethers, esters, ketones, aldehydes, carboxylic acids, amides, cycloalkyls, and aromatic rings.

With regard to n, it is noted that n may equal 1. Accordingly, an embodiment of the present invention includes cyanine dyes having the General Formula VII (i.e. where n = 1)



VII

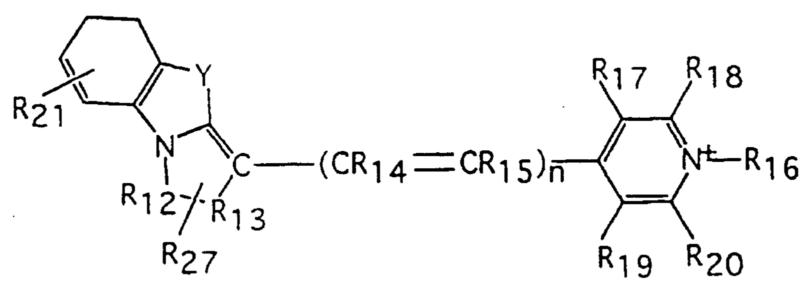
where Y, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20} and R_{21} are as specified above.

With regard to dyes having General Formula VI or VII, Y may be either S or O and is most preferably S.

R_{12} can be an aminoalkyl chain containing a backbone of 3-42 carbons and 1-5 positively charged nitrogen atoms as described in U.S. Patent No. 5,321,130 to Yue, et al. which is incorporated herein by reference. In addition to the positively charged substituents described in U.S. Patent No. 5,321,130, R_{12} is also intended to include aminoalkyl chains including a positively charged cyclic aminoalkyl group having 1-5 positively charged nitrogen atoms.

In a preferred embodiment, R_{12} has the general formula $-R_{28}N(R_{29}R_{30}R_{31})$ where R_{28} is a C_{1-5} alkyl and R_{29}, R_{30}, and R_{31} are each independently a C_{1-10} alkyl.

In an alternate preferred embodiment, R_{12} and R_{13} are taken together to form a 5, 6, 7 or 8 membered ring where the ring includes a positively charged alkyl substituent, more preferably an aminoalkyl chain containing a backbone of 3-42 carbons and 1-5 positively charged nitrogen atoms as described in U.S. Patent No. 5,321,130 to Yue, et al. Dyes of this embodiment may be generally represented by General Formula VIII



VIII

where R_{12} and R_{13} represents the atoms necessary to form a 5, 6, 7 or 8 membered ring and R_{27} is a positively charged substituent, as specified above with regard to R_{12}, which may be attached to any atom used to form the 5, 6, 7 or 8 membered ring as represented by R_{12} and R_{13}. In this regard, these dyes are equivalent to the dyes described above having the General Formula V.

R_{14} and R_{15} may each independently be either hydrogen, C_1-C_{10} alkyl, C_1-C_{10} alkylthio, and are preferably H.

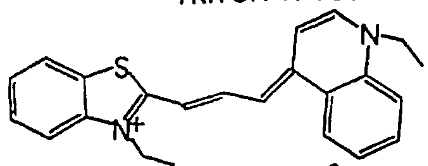
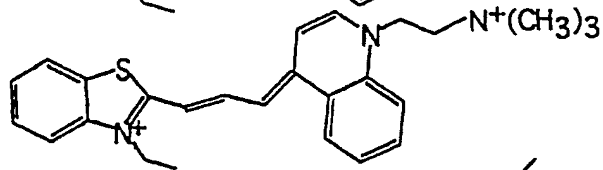
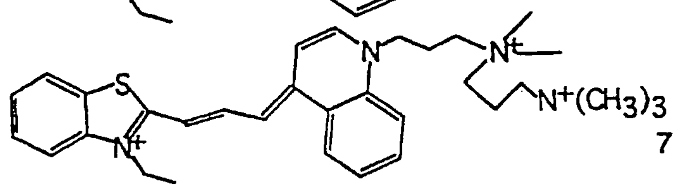
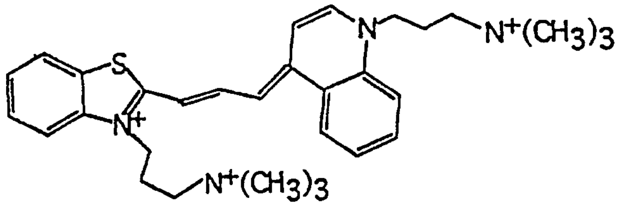
R_{16} may be a C_1-C_{50} alkyl. Since DNA and RNA to which the cyclized cyanine dyes bind contain negatively charged phosphate backbones, it is preferred that R_{16} be substituted with one or more polar substituents. It is most preferred that R_{16} include one or more positively charged atoms in the polar substituent, such as is specified with regard to R_{12} above.

The cyanine dyes according to General Formula VI, i.e., dyes where a positively charged substituent is positioned off the nitrogen of the benzothiazole portion of the dye, provide the advantage over previous cyanine dyes of exhibiting a significantly larger net fluorescence enhancement with DNA than cyanine dyes where a positively charged substituent is positioned at R_{16} alone.

The use of intercalating dyes for staining cell nuclei requires that the dye itself be membrane-permeable or that a membrane permeabilizing step be incorporated into the sample preparation. Methods for enabling charged molecules and very large molecules into cells include the use of chemicals, such as digitonin, freeze-thaw cell lysis steps, or the use of non-ionic detergents such as TRITON X-100. For speed and simplicity, it is preferred to add approximately 9mM TRITON X-100.

The presence of a detergent solution (TRITON X-100) causes significant fluorescence enhancement of the dyes as compared to in PBS buffer. An increase in detergent-enhanced fluorescence (F_{TRITON}/F_{PBS}) has the effect of decreasing the net DNA enhanced fluorescence over detergent-enhanced background fluorescence (F_{DNA}/F_{TRITON}). The detergent-enhanced fluorescence is believed to increase with increasing hydrophobicity.

TABLE 3: Fluorescence Ratios of Dyes in Buffer, TRITON X-100 and DNA Solutions

		E_{Triton}/E_{PBS}	E_{DNA}/E_{PBS}	E_{DNA}/E_{TRITON}
	6 *	94	200	3
	5 *	12	100	8
	7 *	10	70	7
	8	1.8	70	40

* Compounds 5 and 7 are taught in U.S. Patent No. 5,321,130 to Yue, et al.
\# Compound 6 is taught in U.S. Patent No. 4,957,870 to Lee, et al.

Fluorescence enhancement of the dyes upon binding to an excess of DNA was found to be fairly constant regardless of how the quinolinium ring side chain was modified (R_{18}). Advantageously, however, it was found that inclusion of a positively charged substituent off the positively charged nitrogen of the benzothiazole portion of the dye (General Formula VI) causes the dye to exhibit a significantly larger net DNA-enhancement than the positioning of a positively charged substituent at R_{18} alone. As a result, smaller concentrations of nucleic acids can be detected using cyanine dyes having General Formula VI.

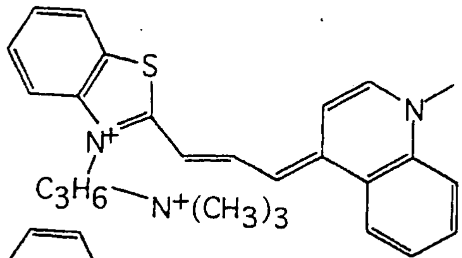
For example, Table 3 compares the fluorescence ratios of dyes in a saline buffer, a detergent (TRITON X-100) and in a DNA solution. Dye solutions (1.0 μM) were prepared in phosphate buffered saline (PBS), in PBS with TRITON X-100 (9mM), and in PBS with double-stranded DNA (100μM).

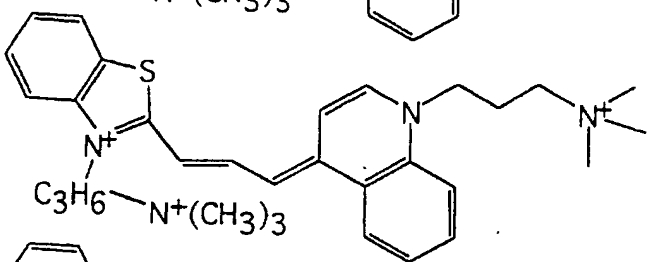
Table 3 shows the effect of various side chains on the fluorescence background in TRITON X-100 (9mM). As illustrated in Table 3, the net DNA enhanced fluorescence over detergent-enhanced background fluorescence (F_{DNA}/F_{TRITON}) was found to be a factor of 5 greater in dye 8 than in dye 7. This result is unexpected since the net charge of 3+ is the same for both dyes 7 and 8. It appears that both the location and quantity of charges affect the fluorescence enhancement of the dyes.

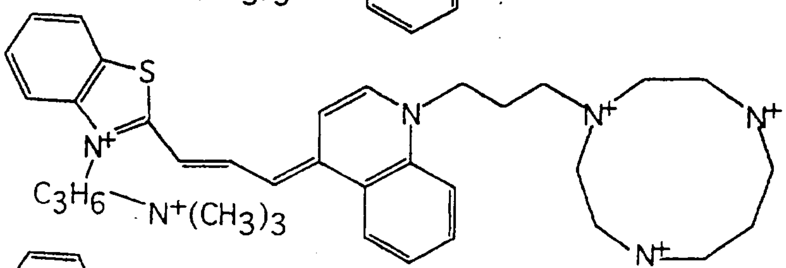
The cyanine dyes according to General Formula VI preferably absorb light at a wavelength of at least about 640 nm, more preferably at least about 649 nm and emit fluorescence at a wavelength of at least about 650 nm, more preferably at least about 663 nm. The cyanine dyes also preferably have a positive Stoke's shift ($\lambda_{Emission}-\lambda_{Abs.}$) of at least 11 nm.

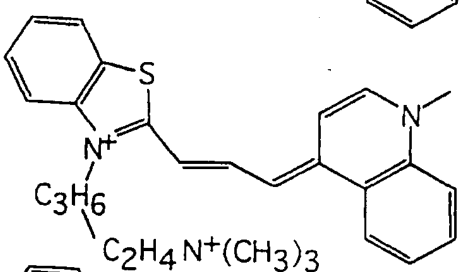
Table 4 provides examples of some of the preferred cyanine dyes having General Formula VI. It should be understood, however, that the dyes listed in Table 4 are intended only to exemplify the cyanine dyes of the present invention and are not intended to be limiting.

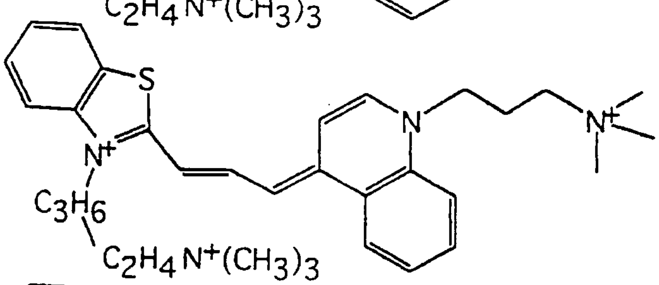
TABLE 4


S
N+
N
C3H6 N+(CH3)3


S
N+
N
N+
C3H6 N+(CH3)3


S
N+
N
N+
N+
N+
C3H6 N+(CH3)3


S
N+
N
C3H6
C2H4 N+(CH3)3


S
N+
N
N+
C3H6
C2H4 N+(CH3)3

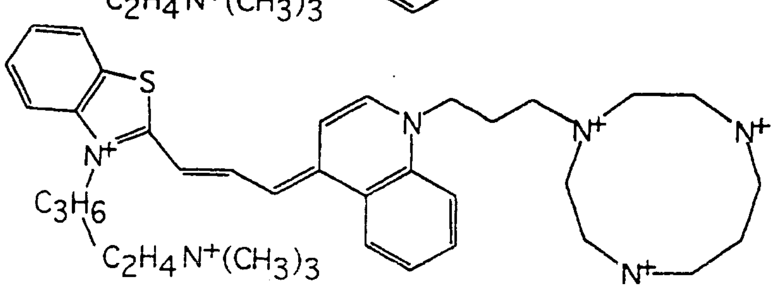
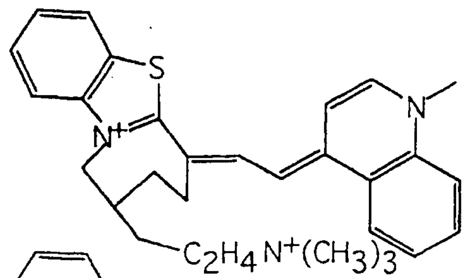
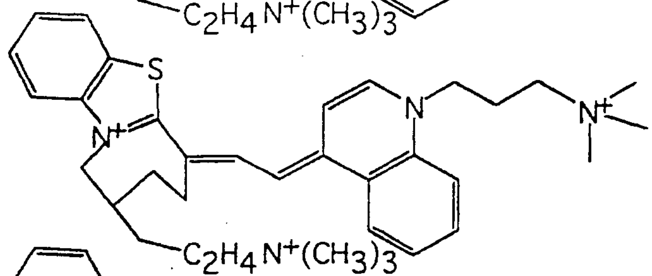
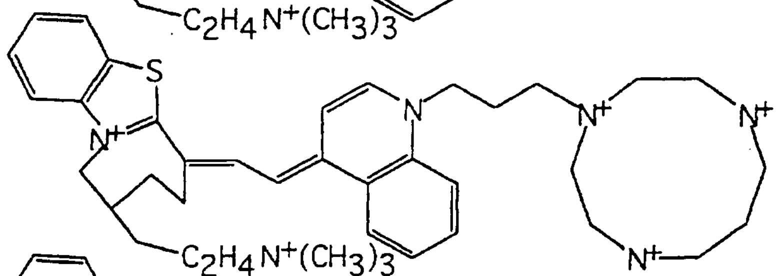
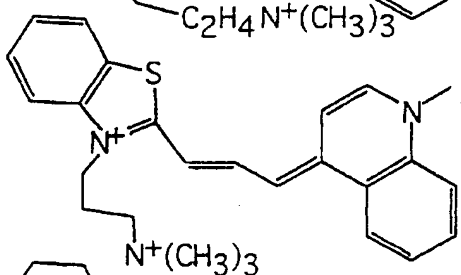

S
N+
N
N+
N+
N+
C3H6
C2H4 N+(CH3)3

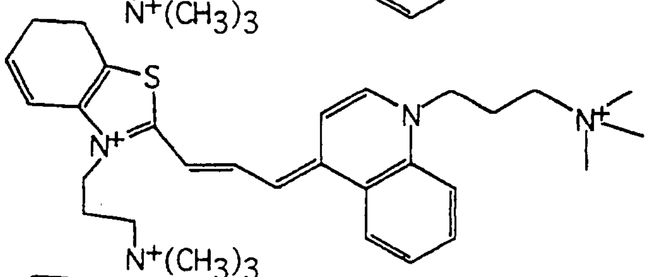
TABLE 4 (cont.)











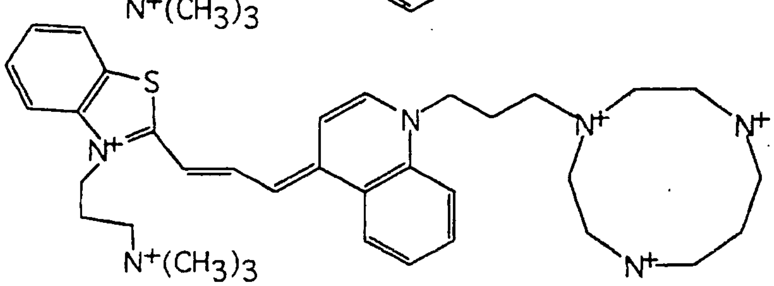
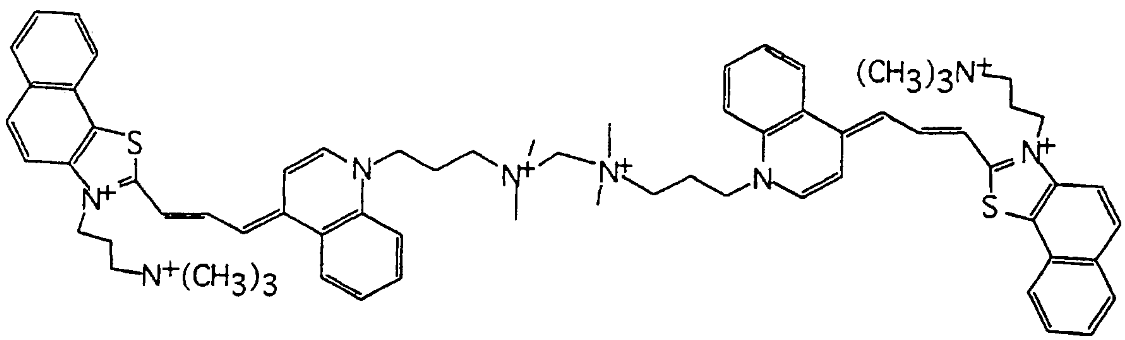
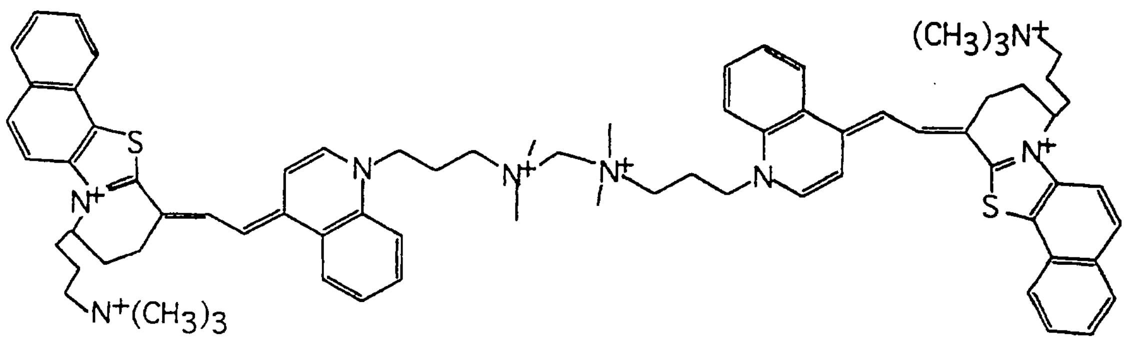


TABLE 4 (cont.)


S
N+
N+(CH3)3
N
N+
N+
N
(CH3)3N+
N+
S


S
N+
N+(CH3)3
N
N+
N+
N
(CH3)3N+
N+
S

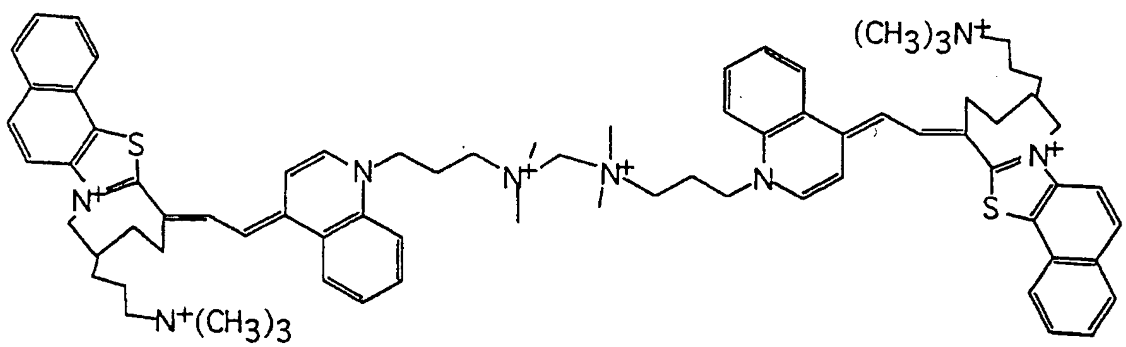
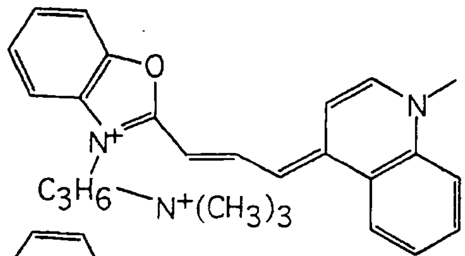
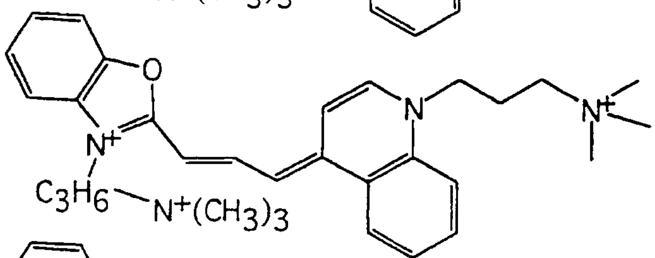
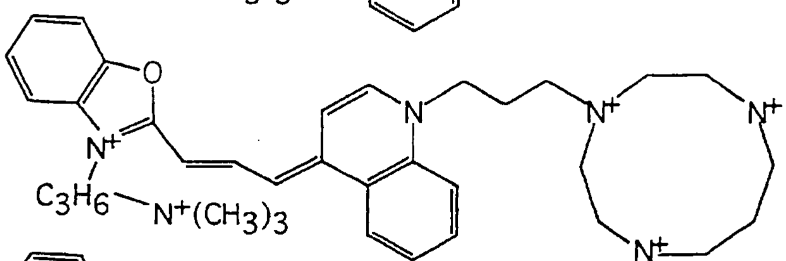
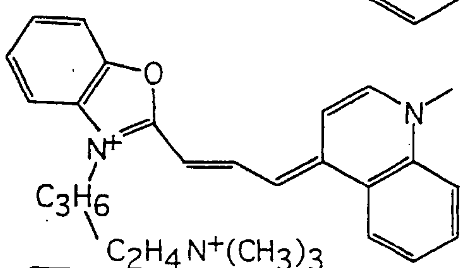

S
N+
N+(CH3)3
N
N+
N+
N
(CH3)3N+
N+
S

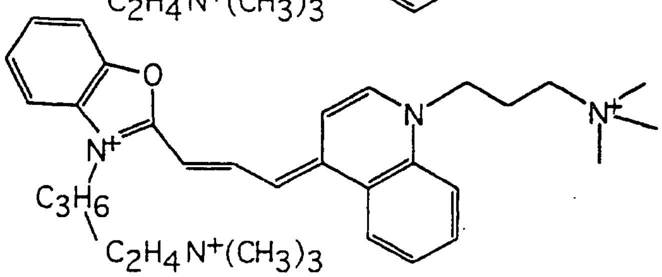
TABLE 4 (cont.)











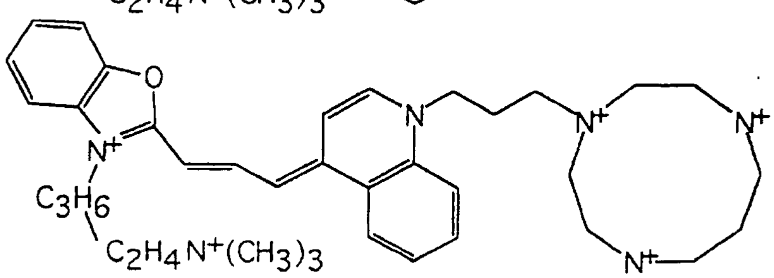
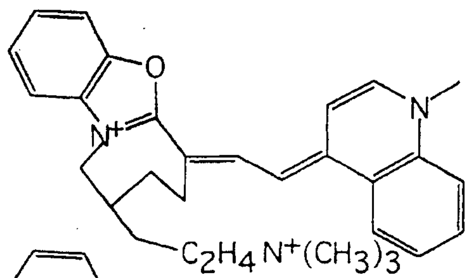
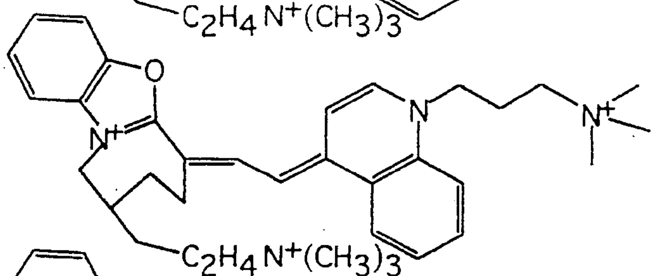
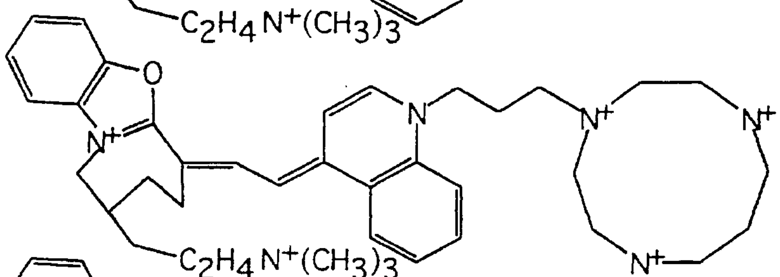
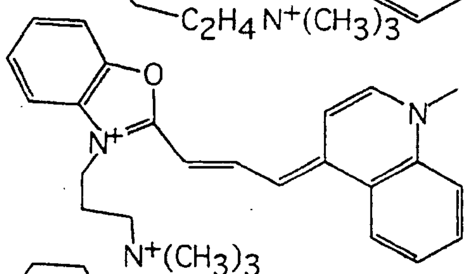


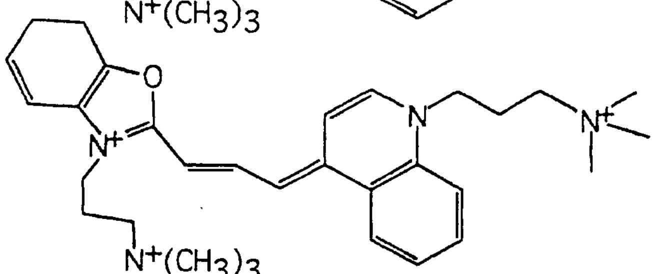
TABLE 4 (cont.)











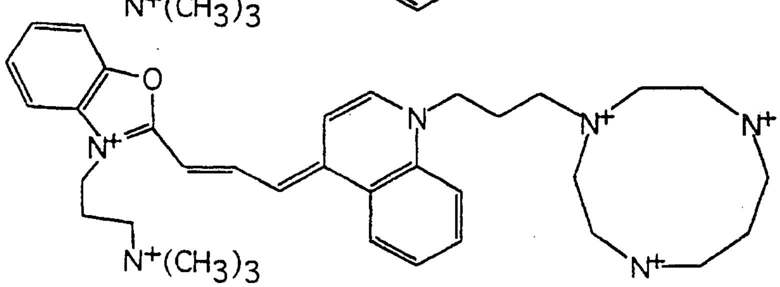
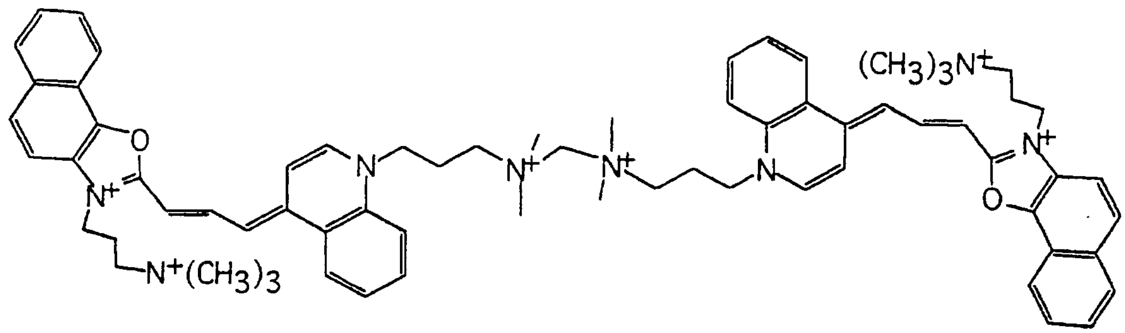
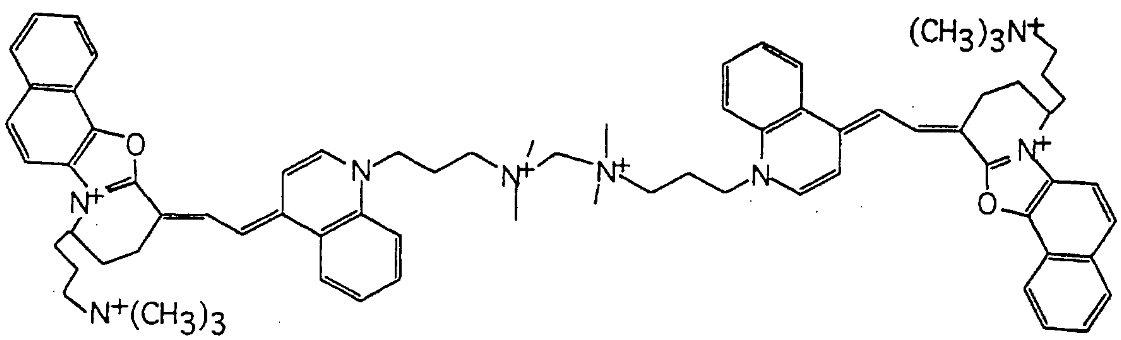
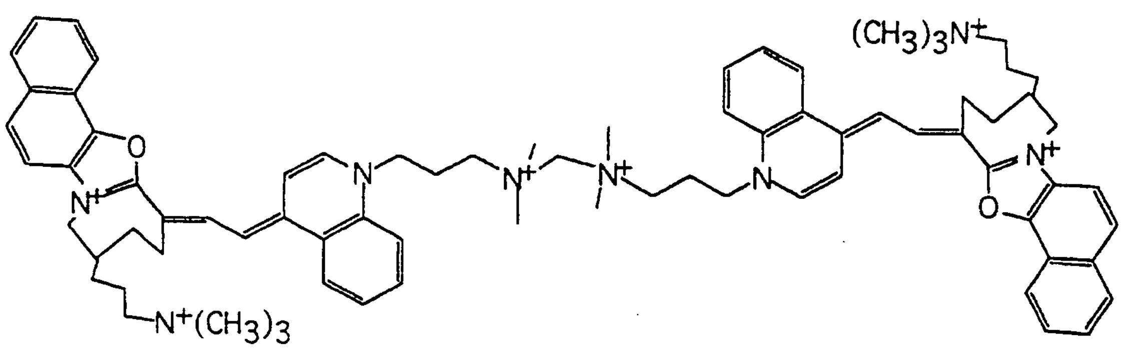


TABLE 4 (cont.)





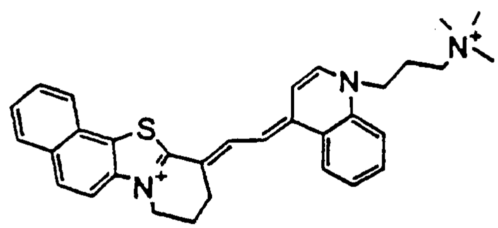


The present invention also relates to the use of the cyanine dyes having General Formulas I, II, IV, V, VI, VII or VIII to form compositions for detecting the presence of nucleic acids in a sample. In general, the compositions include a cyanine dye according to the present invention non-covalently bound to a nucleic acid, i.e., DNA or RNA.

The fluorescence of the cyanine dyes of the present invention significantly increase when bound to a nucleic acid. As a result, it is possible to qualitatively or quantitatively determine the presence of nucleic acids in a sample by monitoring the change in the fluorescence intensity of the dye at a wavelength corresponding to the composition of the dye bound to the nucleic acids. Use of cyanine dyes in general for detecting the presence of nucleic acids in a sample is known in the art. A discussion regarding the use of cyanine dyes to detect the presence of nucleic acids in a sample is provided in U.S. Patent No. 5,321,130 to Yue, et al. which is incorporated herein by reference.

The present invention also relates to a method for detecting nucleic acids by contacting the nucleic acids with a cyanine dye of the present invention. According to the method, a sample of nucleic acids are contacted with a cyanine dye of the present invention in order to form the composition of a cyanine dye non-covalently bound to a nucleic acid sequence. After the dye-nucleic acid sequence composition is formed, the bound dye is exposed to light having a wavelength near an absorbance maximum of the dye when bound to a nucleic acid sequence. The resulting fluorescence emission of the dye is then detected in order to qualitatively or quantitatively determine the presence of nucleic acids in the sample.

Example 1: Preparation of Compound 4



1a. Preparation of 2,3-Tetramethylenenaphth[2,1-*d*]thiazolium Bromide